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Differentiation of a Human Monocytic Cell Line Associated With Increased Production of Rift Valley Fever Virus by Infected Cells

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Rift Valley fever (RVF) virus is a cause of significant human and animal disease in many parts of Africa. In some cases, it causes a hemorrhagic fever, which is frequently fatal. Prior studies have shown that RVF virus productively infects peritoneal macrophages from susceptible rat strains. The U937 human monocytic cell line was used to determine the effect of monocytic cell differentiation on the degree of viral production by cell cultures infected with RVF virus. Differentiation of U937 cells to more mature monocytic cells by phorbol ester resulted in production of 10 times more infectious virions in comparison with undifferentiated cells. These studies imply that monocytic cell differentiation increases permissiveness for RVF virus production.

Key words: monocyte, macrophage, phorbol ester

INTRODUCTION

Rift Valley fever is a frequent cause of disease in domestic animals in sub-Saharan Africa [Peters and LeDuc, 1984]. In humans, it was originally identified as a mild, self-limiting, febrile illness with infrequent complications [Daubney et al, 1933]. Infection with RVF virus is now known to produce a spectrum of illness in humans, ranging from mild disease to fatal hemorrhagic fever [Laughlin et al, 1979; Van Velken et al, 1977; McIntosh et al, 1980].

Rift Valley fever virus has been shown to infect productively peritoneal macrophages isolated from rat strains susceptible to lethal infection by this virus [Rosebrock and Peters, 1982]. Other in vitro virus-monocyte interactions have been investigated, including herpes simplex [Daniels et al, 1978], influenza [Rodgers and Mims, 1981], rubella [van der Logt et al, 1980], Coxsackie [Rager-Zisman and Allison, 1973], yellow fever [Liprandi and

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Walder, 1983], and vaccina [Schultz et al, 1974] viruses. These studies show different susceptibilities of isolated cells, which may influence the outcome of host infection.

In the course of studies on viral hemorrhagic fevers, we infected the U937 human monocytic cell line with RVF virus. This cell line was originally derived from a patient with diffuse histiocytic lymphoma and has since been shown to have characteristics of an undifferentiated monocytic cell, including monocytoid morphology [Sundstrom and Nilsson, 1976]; expression of Fc and complement receptors [Huber et al, 1976]; secretion of lysozyme [Ralph et al, 1976]; presence of cytoplasmic, fluoride-sensitive, nonspecific esterases [Sundstrom and Nilsson, 1977]; and a surface glycoprotein profile compatible with monocyte origin [Nilsson et al, 1981]. Additional evidence of the monocytic nature of U937 cells is the acquisition of features of more mature monocytes. The differentiation can be stimulated by a variety of inducers, such as dimethyl sulfoxide [Kay et al, 1983], vitamin D [Rigby et al, 1984], retinoic acid [Olsson and Breitman, 1982], phorbol ester [Nilsson et al, 1980], and lymphokines [Koren et al, 1979; Moscicki et al, 1983; Pike et al, 1980].

The ability of RVF virus to infect U937 cells and the capability of phorbol ester to induce differentiation of these cells provided an opportunity to determine the effect of cellular differentiation in monocytes on permissiveness for RVF viral infection and production. Undifferentiated U937 cells have been used for studies of Herpes simplex virus type 1 (HSV-1) [Morahan, 1984] and yellow fever virus [Rager-Zisman and Allison, 1973].

MATERIALS AND METHODS

Cells

U937 cells were a gift from Dr. Hillel Koren, Duke University, Durham, NC. Cell cultures were maintained in suspensions in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal calf serum (Gibco, Grand Island, NY), 0.1 mM non-essential amino acids (Gibco), 1 mM sodium pyruvate (M.A. Bioproducts, Hagerstown, MD), and penicillin and streptomycin. Cultures were kept at 37°C in humidified air containing 5% CO_2, and cells were passaged twice weekly at a ratio of 1:5.

Cellular Differentiation

Differentiation of U937 cells to more mature monocytes was achieved by the addition of phorbol 12-myristate, 13-acetate (PMA) (Sigma Chemical Co, St. Louis, MO) to cultured cells. Cell suspensions were washed once by centrifugation at 200 × G and resuspended to 5×10^6 cells/ml. Cells (1.5 ml) were then added to each well of 6-well culture dishes. PMA was added to a final concentration of 150 nM to 3 of the wells.

Experiments of viral growth were expressed in a number of different ways. In initial experiments, virus was added to the cells without further manipulation of the cultures. In subsequent experiments, unattached cells and media were removed. Adherent cells, accounting for 70–90% of the population, were washed, and fresh medium without PMA was added to the wells. The experiment (see Fig. 2) not only called for washing the cells treated with PMA before virus addition, but also required washing the cells, both treated and untreated, after incubation with virus for 60 min at 37°C. The number of attached cells was calculated as the difference between the original number of cells added to each well and the number of unattached cells removed from the wells. Control cultures were prepared from cells not exposed to PMA, and the cell concentration adjusted to that of PMA-treated, attached cells.

ined with a fluorescence microscope and the percentage of infected cells determined. These were graded as follows: 0, 1–9% infected = 1; 10–19% = 2; 20–29% = 3, etc.

RESULTS

After overnight incubation, cultures of U937 cells treated with PMA showed a high degree of cell attachment (70–90%) to the culture wells. In contrast, untreated U937 cells remained in suspension. Cell attachment has been shown to be a marker of cell differentiation [Nilsson et al, 1980].

The effect of cell differentiation on RVF viral replication can be seen in Figure 1. At all time points and for all initial viral concentrations, differentiation was associated with an increase in RVF viral titers of one to several logs.

To determine if PMA exerted an effect on viral production separate from its effect on cell differentiation, three sets of U937 cells were compared. The first set consisted of cells unexposed to PMA. The second set consisted of cells exposed to PMA overnight. The third set consisted of cells exposed to PMA overnight, after which unattached cells and culture media were removed, adherent cells were washed twice in culture medium, and fresh medium without PMA was added to the cultures. Viral infection of these three sets of cells revealed similar levels of viral production in PMA-treated, washed, and unwashed cells, which were significantly higher than those of undifferentiated cells (Table I). Figure 2 represents viral growth after inoculation and washing of treated and untreated cultures. There was obvious, significant, viral production in both the treated and untreated cell cultures. The phorbol ester-treated cells produced significantly ($p < 0.05$) more infective virus at all time points tested.

Immunofluorescence studies of cell cultures at increasing times after viral inoculation revealed higher percentages of infected cells in PMA-treated cultures (Table II). The maximum percentage of immunofluorescent positive cells in these cultures was also reached at earlier time points.

To determine if this effect was specific for the U937 cells or altered other cells that are susceptible to infection with Rift Valley fever virus, Vero cells were treated with PMA for 18 hr and at 150 nM. Figure 3 compares PMA-treated and untreated Vero cells produced in both types of culture at the various moi and sampling times.

TABLE I. U937 Cells-Rift Valley Fever Virus: Effects of PMA on Viral Titer[a]

		Log viral titer	
	Multiplicity of infection	24 h	48 h
	Untreated U937	5.2	5.2
0.1	PMA treated	5.7	6.2
	PMA washed	5.8	6.2
	Untreated U937	3.9	4.2
0.01	PMA treated	4.7	5.1
	PMA washed	4.7	5.3
	Untreated U937	2.8	3.4
0.001	PMA treated	3.6	4.3
	PMA washed	3.7	4.4

[a]U937 cells were divided into three groups and infected with virus at 0.1, 0.01, or 0.001 moi. Following PMA treatment, one group of cells (PMA washed) was washed with culture medium. The other PMA treatment group was infected without removing the PMA-containing medium.

Virus

The Zagazig human (ZH501) strain of RVF virus was used to infect U937 cells [Meegan, 1979]. This strain was initially isolated from a patient with fatal hemorrhagic fever during the 1977 Egyptian epidemic. It was subsequently passed twice in diploid fetal rhesus lung cells (DHS-103) prior to use. The viral stock culture contained 5×10^7 PFU of RVF virus. For infection of cultured cells, virus was diluted to the appropriate multiplicity of infection (moi) in 0.15 ml of culture media. Virus was added to the cultures at moi of 0.01, 0.1, 1, or 10. Cell cultures to which virus had been added were incubated for 30 min at 37°C and then brought to 1.5 ml with fresh medium.

Viral Titers

Media were removed from cell cultures at 4, 24, 48, and 72 hr after addition of virus and assayed for infectious virus. Titers were determined by a plaque assay with African green monkey kidney (Vero) cells. Serial tenfold media dilutions of infectious media were adsorbed to Vero cell monolayers for 60 min, followed by an agarose (Seakem) overlay. These monolayers were allowed to incubate for 72 hr and were then stained with a second agarose overlay containing neutral red (Sigma Chemical Co.). Plaques were counted 24 hr later and viral titers estimated from the counts.

Immunofluorescence Studies

Cultured cells were fixed with acetone on coded slides and stained with a fluorescein-conjugated, hamster, anti-RVF-virus immunoglobulin. The slides were exam-

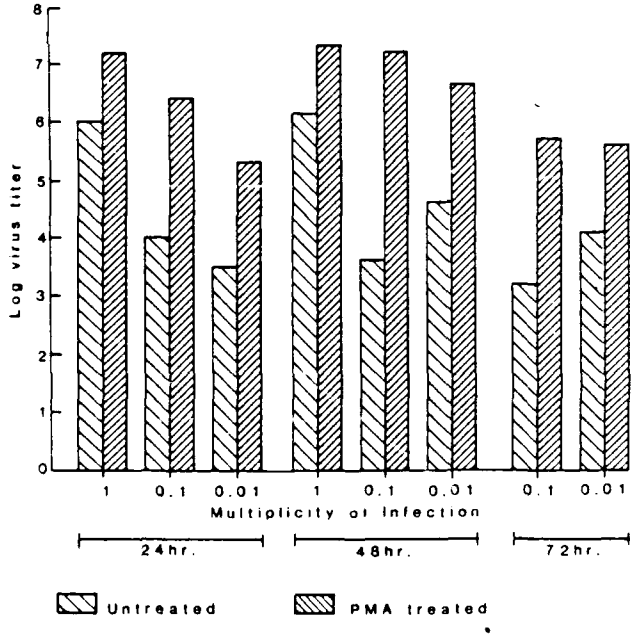

Fig. 1. U937 cells were infected with RVF virus. Cultures were treated with PMA for 10 hr prior to infection. Samples for virus titers were taken at the [illegible] shown.

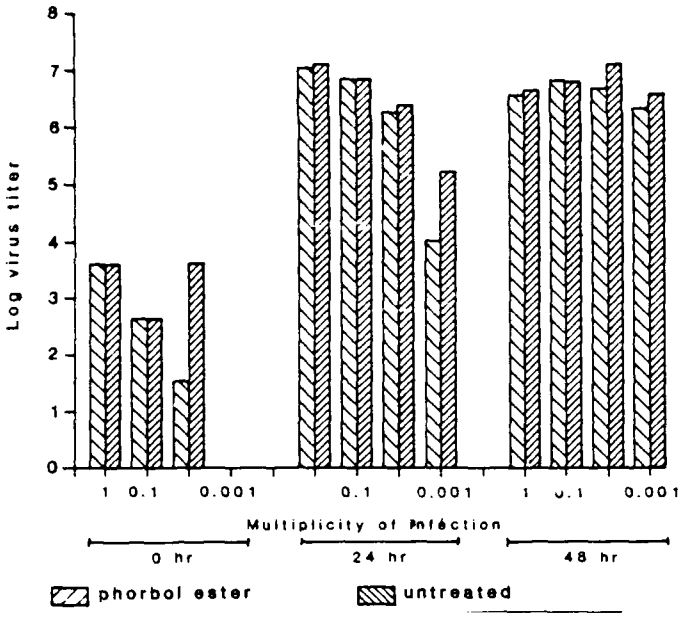

Fig. 3. Confluent Vero cells were infected with RVF virus. Cultures were treated with PMA for 18 hr prior to infection. Samples for virus titers were taken at times shown.

latter is based on the calculation that the increase in percentage of infected cells in PMA-treated cultures can account for only a small part of the rise in viral titers in these cells, and not for the observed increase of 10- to 100-fold. The evidence is inconclusive, however, because of the potential for continued replication of undifferentiated U937 cells.

The possibility that residual PMA in the culture medium might itself have affected viral production was considered and rejected because cells that were washed after the PMA treatment did not differ from unwashed cells in their ability to support viral production. PMA is removed from the cell surface following the addition of fresh medium as evidenced by renewed ability of the cells to bind PMA [Cooper et al, 1982].

Differentiation of U937 cells by phorbol esters is a membrane-mediated event [Cooper et al, 1982]. Additionally, the cell membrane itself undergoes a number of changes [Nilsson et al, 1980; Rouis et al, 1984], one of the most graphic of which is manifested by the change from a nonadherent to an adherent population of cells. The first step in virus-monocytic cell interaction, viral attachment, is another membrane-mediated event [Shif and Bang, 1970]. An increase in the percentage of PMA-treated U937 cells infected by RVF virus may simply reflect a differentiation-induced increase in viral adherence to cell membranes. This, however, may account for only a portion of the increased production of virus by PMA-treated cells. Intracellular events may play a prominent role in the augmentation of viral production in these cells, in which case changes in genomic expression, which occur as a consequence of cellular differentiation and maturation, are likely to contribute to the augmentation.

The unrelated Pichinde virus was tested for infectivity of U937 cells either in

PMA-treated or untreated cultures (data not shown). Viral production in these cultures could not be demonstrated and implies that the PMA does not allow nonspecific viral entry but augments viral production in PMA susceptible cells. Also, the susceptibility of a cell line to infection is not sufficient for this augmentation, but could be sensitive to the effects of the tumor promoter as shown with Vero cells. In the instance of the U937 cells, the susceptibility to differentiation induction by PMA may be the important characteristic.

The degree of cellular differentiation in monocytic cells appears to be an important determinant of the susceptibility of these cells for infection by viruses. The present report documents the importance of differentiation of early monocytic precursors to more mature monocytes in the modulation of cell-virus interactions. Studies with HSV-1 have demonstrated the importance of cellular changes (in vitro) beyond the circulating monocyte stage [Rosebrock and Peters, 1982; Linnavuori and Hovi, 1981]. HSV-1 produces only limited amounts of infectious virus in newly isolated human monocytes. After incubation in culture, however, these cells can be productively infected. Similar findings have been reported for rubella virus [Rodgers and Mims, 1981].

Alterations that occur in monocytic cells and that transform them into fixed macrophages in various organs also affect the ability of the cells to support viral infection and production. Rabbit alveolar macrophages are killed by HSV-1 virus, but are not productively infected [Plaeger-Marshall et al, 1982]. In contrast, peritoneal exudate cells in these same animals support viral replication. Precisely opposite results have been observed in mice with influenza virus [Daniels et al, 1978].

The importance of genetically mediated variability in resistance of monocytic cells to viruses is well documented [Mogenson, 1979]. In the case of RVF virus, there are marked differences in the susceptibility of peritoneal macrophages to productive infection when isolated from different rat strains [Schultz et al, 1974]. Studies of murine hepatitis virus type 2 in strains of mice have revealed complete agreement between animal and macrophage susceptibility to infection with this virus [Bang and Warwick, 1960].

The importance of age-related changes in resistance of monocytic cells to viruses has also been studied extensively [Morahan, 1984]. In this respect, models such as HSV-1 infection in mice [Hirsch et al, 1970] have provided much useful information.

The fact that U937 cells can be induced to differentiate to more mature monocytic cells by a number of different agents and the demonstration that this in turn has a marked effect on viral production in these cells provide a useful model for studying the determinants of susceptibility and resistance of monocytic cells to viral infection and production.

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